www.sciencemag.org/cgi/content/full/1116261/DC1

Supporting Online Material for

Stem-Cell Homeostasis and Growth Dynamics Can Be Uncoupled in the *Arabidopsis* Shoot Apex

G. Venugopala Reddy and Elliot M. Meyerowitz*

*To whom correspondence should be addressed. California Institute of Technology, Division of Biology, MC 156-29, 1200 East California Boulevard, Pasadena, CA 91125, USA. E-mail: meyerow@its.caltech.edu

Published 6 October 2005 on *Science* Express
DOI: 10.1126/science.1116261

This PDF file includes

***Science* Supporting Online Material**

Stem-Cell Homeostasis and Growth Dynamics Can Be Uncoupled in the *Arabidopsis* Shoot Apex

G. Venugopala Reddy and Elliot M. Meyerowitz*

*To whom correspondence should be addressed. California Institute of Technology, Division of Biology, MC 156-29, 1200 East California Boulevard, Pasadena, CA 91125, USA. E-mail: meyerow@its.caltech.edu

Materials and Methods

Transgenic lines and growth conditions.

The generation of *35S*::*YFP29-1* plants has been described (*S1*). The p*CLV3*::*mGFP5-ER* construct was generated by introducing a PCR-amplified *mGFP5-ER* (a gift from Jim Haseloff) fragment into the BamHI site of pBu (a gift from Rudiger Simon), a vector carrying both the upstream and the downstream fragments flanking the *CLV3* ORF (*S2*). The DEX-inducible form of the *35S*::*GR:LhG4-N* construct (a gift from Ian Moore) was introduced into Landsberg *erecta* (L*er*) plants to obtain stable kanamycin-resistant transformants. The *6XOP*Ω::*CLV3*dsRNAi construct was generated by introducing a BamHI and PstI fragment from the coding region of the construct, which makes a foldback *CLV3* RNA described in (*S3*), into the *6XOP*Ω promoter housed in the pPZP vector and then introduced into L*er* plants to obtain gentamycin-resistant transformants. The RT-PCR analysis of the *CLV3* gene was carried out with the primers, CLV3RTF: AGTTTCTATATTTCTCTCTGTATC and CLV3RTR: GAAATAATTTAAAGCAACAAGAGA. RNA in situ hybridization experiments were carried out according to the protocol posted at http://plantlab.caltech.edu/html/protocols.html, by utilizing RNA probes corresponding to the entire coding regions of *mGFP5-ER* or *WUS*.

The DEX treatment was imposed in different ways depending on the experimental requirements. For the initial phenotypic analysis, seeds were germinated on 10 μM DEX containing MS-agar plates, the seedlings were transferred to soil and watered with DEX solution every alternate day until bolting. For transient induction of *CLV3* RNAi and live imaging, refer to the following section. All plants grown either on soil or on plates were maintained in continuous light and at 22°C.

Live imaging and microscopy

Seeds were germinated on MS-agar plates and allowed to grow for 7 days before they were transferred into clear plastic boxes containing MS-agar. The plants were maintained in aseptic conditions until bolting (16-18 days after germination). Upon bolting, when the shoot apex emerged out of the rosette, the plants were either treated with DEX (10 μM DEX and 0.015% Silwet L-77) or mock-treated (0.015% Silwet L-77 and the relative proportion of ethanol used for dissolving DEX) by placing a droplet on top of the SAM.

After every imaging session (12-hour or 24-hour intervals) a drop of DEX or mock solution was applied to the SAM. However, the Silwet was included only for the first application. The older floral buds were carefully removed or spaced out so as to expose the SAM. Plants were imaged by using a Zeiss LSM 510 META upright confocal microscope using a 63× achroplan lens. GFP and YFP were stimulated with an argon laser at 488 nm and 514 nm. Emission wavelengths were filtered by using band-pass (BP505-530nm) and long-pass (LP530) filters to collect GFP and YFP signals, respectively. The simultaneous acquisition of double-labeled images of GFP and YFP was done with a multi-track option. The Z-stacks were reconstructed in three dimensions by using the Zeiss LSM3.2 software, and the images were assembled in Adobe Photoshop7.0. In some experiments, FM4-64 (50 μg/mL) was applied directly onto the SAM 15-30 min prior to imaging.

References

S1. G. V. Reddy, M. G. Heisler, D. W. Ehrhardt, E. M. Meyerowitz, *Development* **131**, 4225 (2004).

S2. U. Brand, M. Grunewald, M. Hobe, R. Simon, *Plant Physiol*. **29**, 565 (2002).

S3. C. F. Chuang, E. M. Meyerowitz, *Proc. Natl. Acad. Sci. U.S.A.* **97**, 4985 (2000).

Supplementary Figures

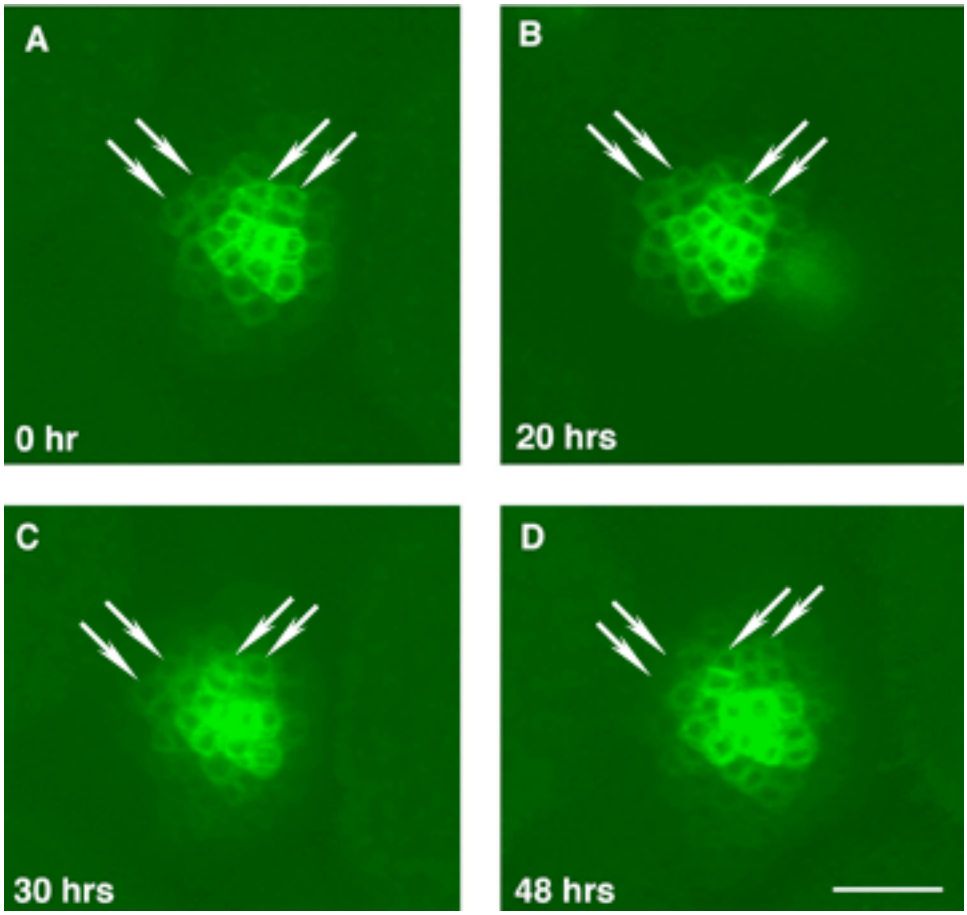

Figure S1. Temporal changes in p*CLV3* expression in wild type. (A-D) Reconstructed 3-D views of the L1 layer of the SAM expressing p*CLV3*::*mGFP5-ER* at different time intervals. Total elapsed time is marked on each panel. Note subtle changes in the expression domain with individual cells at the periphery of the domain beginning to lose GFP expression with time. The cells in the center of the expression domain maintain their expression levels. Arrows point to the same cells in images acquired at successive intervals. Scale bar 20μM.

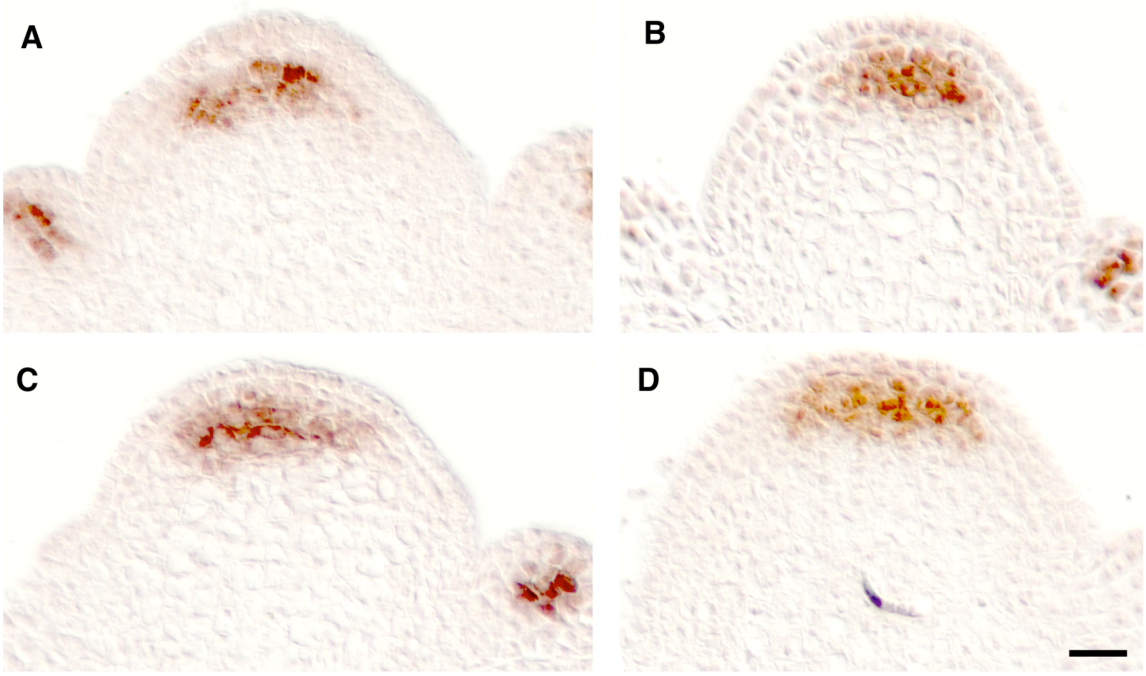

Figure S2. *WUS* expression pattern upon DEX treatment. (A-D) Longitudinal sections of four different SAMs, showing the *WUS* RNA expression domain after treatment with DEX for a period of 7 days after bolting. Compare with mock treated SAM in Fig. 1 I. In DEX treated SAMs the *WUS* expression domain has expanded laterally, and the domain appears patchy, consisting of cells with variable levels of expression. Scale bar 20μM.